Effect of microbial inoculants on *Albizia saman* germination and seedling growth

B. M. Khan[1], M. K. Hossain[1] and M. A. U. Mridha[2]
[1]*Institute of Forestry and Environmental Sciences, University of Chittagong, Chittagong-4331, Bangladesh.*
[2] *Department of Botany, University of Chittagong, Chittagong-4331, Bangladesh*

Abstract: Microbial Inoculants as Effective Microorganisms (EM) were applied to find out their effects on germination and seedling growth of *Albizia saman* in the nursery. The seedlings were grown in a mixture of sandy soils and cow dung (3:1) kept in polybags. The EM solution at different concentrations (0.1%, 0.5%, 1%, 2%, 5% and 10%) was incorporated before and after a week of sowing seeds. Germination and physical growth parameters, including shoot and root length, vigor index, collar diameter, leaf number, fresh and dry weight of shoot and root and total biomass increment over the control were measured. The nodulation status influenced by EM was also observed along with the estimation of chemical parameters like chlorophyll a, chlorophyll b and carotenoid. Both germination and the measured physical growth parameters were found significantly ($P<0.05$) higher in seedlings treated with different concentrations of EM solution in comparison to the control. Maximum growth was found at 2% followed by 1% EM solution. Nodulation was higher at 0.1% concentration but it normally decreased with the increase of concentrations. Although there were a higher amount of pigments in leaves of the treated seedlings than of the control, the variations recorded with respect to chlorophyll a, b and carotenoid were not significantly higher in most of the treatments. Treated seedlings showed variable results along with the increment of EM applications and most of the parameters showed best results at the medium range of concentrations. The study indicates that the Microbial Inoculant (EM) technology might be useful to improve the growth of seedlings in the nursery. This also indicates that the associated beneficial organisms along with the polybag soils might be of value in improving the degraded soil or poor field soil for better nutrient and water uptake during the initial growth of transplanted seedlings.

Keywords: *Albizia saman* (Jack.) Merr.; Microbial Inoculants (EM); Germination; Seedling growth; Leaf pigment; Nodulation status
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Introduction

Albizia saman (Jack.) Merr. locally known as Rain tree (Family: Leguminosae) is a middle-sized to large, fast growing, multipurpose semi-deciduous tree species, which can grow well in fairly drained, neutral to slightly acidic heavy soil texture. It is native to Central America, West Indies and Guyana and widely distributed in the tropical forests of Asia (Das and Alam 2001). The species has been getting priority in Agroforestry, Community Forestry, Social Forestry, Village and plantation programmes of Bangladesh as well as other several tropical countries of the world. Its timber is hard, brown and used for furniture, carvings, paneling, boat building and construction work (Luna 1996). It coppices well and often lopped for firewood and fodder (Das and Alam 2001). Many organizations are producing *A. saman* seedlings in their nurseries for demanding plantation programmes. Though *A. saman* is used for wide range of purposes, the initial growth potential under the influences of microbial inoculants, *e.g.* Effective Microorganisms (EM) was not studied.

The objective of this study is to observe the effectiveness of EM inoculants on germination and the growth of *A. saman* seedlings and to determine the best concentration of EM solution for maximum development of the seedlings in the nursery. The microbial inoculants (Effective Microorganisms or EM) used in this study was developed at the University of Ryukyus, Okinawa, Japan in the early 1980's by Professor Teruo Higa. The main species comprising EM are Lactic acid bacteria, photosynthetic bacteria, beneficial fungi, yeast and ray fungi (Kyan *et al*. 1999). The naturally occurring microorganisms are combined in the inoculant manufacturing process and can survive in the inoculant liquid at pH 3.5 or below. The densities of most of the above mentioned microbes are in the range of 1×10^6 to 1×10^8 per ml (Xu 2000), which can be applied as inoculants to increase the microbial diversity of soils and plants. The inoculation of EM cultures to the plant ecosystem can improve the photosynthesis and fruit yield of plants (Xu 2000; Wang *et al*. 2000).

Materials and methods

The experiment was carried out in the nursery of the University of Chittagong, Chittagong, Bangladesh (Lies approximately at the intersection of 91º50′E longitude and 22º30′N latitude). The seeds of *A. saman* were collected from Bangladesh Forest Research Institute. The soils, collected from the degraded hills of the University Campus, was sieved well (<3 mm) and mixed with decomposed cow dung in a ratio of 3:1. The brown hill soils are sandy loam to sandy clay loam, moderately to strongly acid and poorly fertile with pH<5.5, organic matter<2.0%, CEC<10 me/100g and BSP<40% (Osman *et al*. 2001). Polybags of 15 cm ×10 cm in size were filled with the prepared mixtures, and a layer of coir (1 cm) was provided in each of the polybags as a top layer of the polybag media to reduce the evaporation and to supply a source of organic matter. A Complete Randomized Design (CRD) was adopted for a total of seven treatments including a control treatment and three replications for each treatment (Con-

Biography: Bayezid Mahmud Khan (1976-) male, Assistant Professor in the Institute of Forestry and Environmental Sciences, University of Chittagong, Chittagong-4331, Bangladesh. E-mail: bmkhan2004@yahoo.com
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trol treatment also included 3 replications) with 20 polybags for each replication (e.g. there were 60 polybags for each treatment and a total of 420 polybags for the whole experiment). Seeds sown in polybags without EM, treated as control and the remaining polybags were treated with EM solutions at 0.1%, 0.5%, 1%, 2%, 5% and 10% concentrations, respectively. Fifty mL EM solution was mixed with the soils before one week of sowing seeds and another 50 mL was applied after one week of sowing seeds in each of the polybags. Three seeds were sown in an individual polybag to observe the influence of EM on germination (e.g. a total of 180 seeds for per treatment and 1 260 seeds for whole experiment for germination test) and after completion of germination only one seedling (Best one) per polybag was maintained to observe the physical growth parameters and chlorophyllus status of the leaves of seedlings. Partial shade and covering was provided over the nursery to protect the seedling from strong sunlight and rains.

Germination was recorded daily from the date of seed sowing and continued up to germination of the last seed. The seedlings were allowed to grow altogether for three months from the time of seed sowing. After three months, five seedlings from each replication of a treatment were selected for measuring physical parameters of shoot and root length, collar diameter, leaf number, fresh and dry shoot and root weight, nodule number and their fresh and dry weight. For determining the seedling dry weight, shoots and roots was oven dried at 70ºC until the constant weight was obtained. Vigor index and total dry biomass increment (%) were also calculated.

The pigment contents (Chlorophyll a, chlorophyll b and carotenoid) in the fresh leaves of seedlings of different treatments were determined with the leaves collected from the second, third and fourth from the top. Ten leaf discs were cut with a cork borer (inside diameter 5 mm), weighed immediately after cutting and dipped in 5 mL100% acetone in test tube with stopper. After 24 hours of incubation, the supernatant colored solution from the top was decanted carefully in 25 mL volumetric flask. The leaf discs were then crushed with a blunt glass rod gently and 5 mL fresh acetone was added in the test tube and left for 15 min. Then the supernatant solution from the top was again decanted into the same volumetric flask very carefully, avoiding the fragmented plant tissues. The process was repeated until the leaf fragments became colorless. Finally the volume was made up to 25 mL with fresh acetone and measurement was taken immediately after the preparation of solution. The measurements of chlorophyll a, chlorophyll b and carotenoid were made at 662 nm, 644 nm and 440.5 nm, respectively, with a spectrophotometer (Spectronic-20). The concentrations of pigments in the extract were calculated by following the formula of Wettstein (1957).

$$\text{Chlorophyll a} = \frac{(9.784 \times E662 - 0.990 \times E644) \times V \times d}{1000 \times FW},$$

mg/L

$$\text{Chlorophyll b} = \frac{(21.426 \times E644 - 4.650 \times E662) \times V \times d}{1000 \times FW},$$

mg/L

$$\text{Carotenoid} = \frac{[4.695 \times E440.5 - 0.268(\text{Chlorophyll a} + \text{Chlorophyll b})] \times V \times d}{1000 \times FW}$$

mg/L

Where, V = Total volume (25 ml); d = Dilution factor; FW = Fresh weight of leaf disc.

All the data were analyzed statistically by using the computer software package SPSS and they were subjected to analysis by DMRT.

Results and discussion

Germination and morphological growth parameters of seedling

The effects of EM on the germination and morphological growth parameters of seedlings like, shoot length, root length, total length, collar diameter and leaf number are shown in Table 1. The highest germination (71%) was observed in 2% EM solution followed by 68% germination in 5% and 66% germination in 1% EM concentration. The lowest germination (57%) was found in the control treatment. The highest shoot growth (37.9 cm) was observed in 2% EM solution whereas the highest root growth (21.2 cm) was found in 1% EM. Collar diameter was highest (8.23 mm) in 5% EM solution and was significantly ($P<0.05$) different from that of the control. Vigor index was highest (4182) in 2% EM solution followed by 5% and 1% concentrations and was significantly ($P<0.05$) different from that of the control (Table 1).

Table 1. Effect of Effective Microorganisms (EM) on germination, shoot and root length, vigor index, collar diameter and leaf number of *A. saman* after 3 months of sowing seeds

Concentration of EM (%)	Germination (%)	Length (cm)			Vigor index	Collar dia. (mm)	Number of leaf
		Shoot	Root	Total			
Control	57 b *	29.6 c	17.6 b	47.2 b	2690 b	7.12 b	15.2 b
0.1	62 b	33.8 b	18.2 b	52.0 ab	3224 ab	7.37 a	15.8 b
0.5	63 b	35.3 ab	19.5 ab	54.8 ab	3452 ab	7.45 a	17.2 a
1	66 a	36.7 a	21.2 a	57.9 a	3821 a	7.98 a	18.2 a
2	71 a	37.9 a	21.0 a	58.9 a	4182 a	8.16 a	18.4 a
5	68 a	37.7 a	19.9 ab	57.6 a	3917 a	8.23 a	17.6 a
10	59 b	32.4 b	18.7 ab	51.1 ab	3015 ab	7.74 a	16.2 a

*- Means followed by the same letter (s) in the same column do not vary significantly at $P<0.05$, according to Duncan's Multiple Range Test (DMRT).

Fresh and dry matter production

Fresh and dry matter production such as shoot and root fresh weight, total fresh weight, shoot and root dry weight and total dry weight are tabulated in Table 2. Both fresh and dry shoot weights were maximum (9.22 g and 3.82 g, respectively) in 2% concentration of EM solution and were significantly (P<0.05) different from those of the control. Similarly, both fresh and dry root weights were maximum (3.13 g and 1.53 g, respectively) in 1% EM solution and were significantly (P<0.05) different from those of 0.1%, 10% EM and the control. In all the cases, the lowest growth was observed in control treatments (Table 2). Total dry biomass increment (%) was highest in 1% EM solution followed by 2% and 5% concentrations and was positive for all the treatments compared to the control. Increased biomass production might be due to the better root development (Lim *et al*., 1999) and by the production of growth enhancing components such as IAA (Indole Acetic Acid) and gibberellins, which may have positive influence on plant growth (Chowdhury *et al*. 1994).

Table 2. Effect of Effective Microorganisms (EM) on shoot and root fresh and dry weights of *A. saman* after 3 months of sowing seeds.

Concentration of EM	Fresh weight (g)			Dry weight (g)			Total dry biomass increment
(%)	Shoot	Root	Total	Shoot	Root	Total	(%)
Control	7.41 b *	2.20 b	9.61 b	3.13 b	1.14 b	4.27 b	00.00
0.1	7.63 b	2.51 b	10.14 ab	3.21 b	1.23 b	4.44 b	+3.98
0.5	7.92b	2.90 a	10.82 ab	3.54 a	1.50 a	5.04 a	+18.03
1	8.85 a	3.13 a	11.98 a	3.76 a	1.53 a	5.29 a	+23.90
2	9.22 a	2.84 a	12.06 a	3.82 a	1.46 a	5.28 a	+23.65
5	8.64 a	2.72 a	11.36 a	3.67 a	1.42 a	5.09 a	+19.20
10	7.73 b	2.45 b	10.18 ab	3.35 b	1.21 b	4.56 b	+6.79

*- Means followed by the same letter (s) in the same column do not vary significantly at P<0.05, according to Duncan's Multiple Range Test (DMRT).

Application of EM solution can play a role in enhancing germination, growth and yield of various agricultural crops and vegetables (Iwaishi 2000; Shin *et al.* 1995; Zacharia 1995). EM solution with organic fertilizers and other chemicals is also reported to enhance germination, growth and yield of different agricultural crops (Anuar *et al*. 1995; Xu 2000), but the influence of EM on forest crops has not been studied widely (Khan *et al.* 2002). From the present study it has been observed that soil amended with different concentrations of EM provided higher growth, but increased EM concentration (>2%), seedling growth gradually decreased. The findings are in accordance with Mridha *et al*. (1999), that is, lower EM concentration enhanced germination, whereas higher concentration of EM decreased germination, which may be due to toxicity of higher concentrations of EM.

Nodulation status of seedling

Nodulation status of seedlings is presented in Table 3. Nodule number was highest (151) in 0.1% concentration of EM solution followed by 141 in control, 138 in 0.5% and 133 in 2% concentrations. The lowest nodule number was 109 in 10% EM solution. Both fresh and dry weights of nodules were maximum (1.55 g and 0.56 g, respectively) at 0.1% concentration and lowest (1.12 g and 0.37 g, respectively) in 10% concentration of EM solution. The rate of nodule increment was positive in case of 0.1% EM solution and negative for all other treatments compared with the control. Nodule fresh and dry weight increment rates were positive in 0.1% and 0.5% concentrations and negative for other treatments compared with the control. These results support the finding of Thach *et al.* (1999), that is, the increase in number of nodule in soybean roots was not significant due to application of EM.

Pigments concentration of fresh leaf

Effects of EM on the concentration of leaf's pigments (Chlorophyll a, chlorophyll b and carotenoid) were determined and the results are presented in Table 4. Chlorophyll a and chlorophyll b were highest (62.72 $mg{\cdot}L^{-1}$ and 17.85 $mg{\cdot}L^{-1}$, respectively) in 2% concentration of EM solution and lowest (38.75 $mg{\cdot}L^{-1}$ and 12.82 $mg{\cdot}L^{-1}$, respectively) in the control. Carotenoid was highest (43.52 $mg{\cdot}L^{-1}$) in 1% EM solution followed by 5% and 2% solutions. Total pigment was highest (119.99 $mg{\cdot}L^{-1}$) in 2% EM solution and was significantly (P<0.05) different from those in most of the treatments including the control. Total pigments increment (%) was positive for all the treatments compared with ~~to~~ the control. The present results are in agreement with Xu (2000), Wang *et al*. (2000) and Mridha *et al.* (2002), that is, EM applied with organic fertilizers has shown a promotion of root growth and activity, and enhancements of photosynthetic efficiency and capacity, which resulted in increased yields.

Table 3. Effect of Effective Microorganisms (EM) on nodule number and fresh and dry weights of *A. saman* after 3 months of sowing seeds.

Concentration of EM	Nodule					
(%)	Number	Weight (g)		Number increased/ decreased	Weight increased/ decreased (%)	
		Fresh	Dry	(%)	Fresh	Dry
Control	143 a *	1.46 a	0.49 a	00.00	00.00	00.00
0.1	151 a	1.55 a	0.56 a	+5.59	+6.16	+14.29
0.5	138 a	1.47 a	0.50 a	-3.50	+0.68	+2.04
1	131 a	1.38 a	0.47 a	-8.39	-5.48	-4.08
2	133 a	1.37 a	0.46 a	-6.99	-6.16	-6.12
5	127 b	1.29 b	0.43 b	-11.19	-11.64	-12.24
10	109 b	1.12 b	0.37 b	-23.78	-23.29	-24.49

*- Means followed by the same letter (s) in the same column do not vary significantly at P<0.05, according to Duncan's Multiple Range Test (DMRT).

Table 4. Effect of Effective Microorganisms (EM) on pigment concentrations in fresh leaves of *A. saman* after 3 months of sowing seeds.

Concentration of EM (%)	Pigment concentration of leaf (mg/L)				Total pigment increment (%)
	Chlorophyll a	Chlorophyll b	Carotenoid	Total	
Control	38.75 c *	12.82 b	28.67 b	80.24 c	00.00
0.1	46.42 b	13.58 ab	37.94 ab	97.94 b	+22.13
0.5	43.93 b	15.78 a	36.99 ab	96.70 b	+20.58
1	55.45 ab	14.56 ab	43.52 a	113.53 a	+41.61
2	62.72 a	17.85 a	39.42 a	119.99 a	+49.69
5	57.36 a	15.25 ab	41.58 a	114.19 a	+42.43
10	52.05 ab	16.21 a	34.97 ab	103.23 b	+28.74

*- Means followed by the same letter (s) in the same column do not vary significantly at P<0.05, according to Duncan's Multiple Range Test (DMRT).

From the present findings it can be concluded that low concentration of EM solution (up to 2%) may be used for getting maximum seed germination and seedling growth of *A. saman* and may influence better growth and yield in the field. However, further evaluation of EM on *A. saman*, may be applied to confirm the beneficial effects on germination, seedling development in the nursery and out plantings in the field.

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